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71 Applicant: COMTECH RESEARCH UNIT LIMITED, Bank of Bermuda Building, Hamilton 5-31 (BM)

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72 Inventor: Layton, Derek Geoffrey, 4 Rutherford Road, Cambridge (GB)  
Inventor: Pettigrew, Robert Martin, Pound Cottage High Street, Foxton Cambridgeshire (GB)  
Inventor: Smith, Alan Martin, 68 Greengage Rise, Melbourn Royston Herts (GB)  
Inventor: Petty-Saphon, Satham, Woodnook, Rickling Saffron Walden Essex (GB)  
Inventor: Fisher, John Horace, 27 Bridge Street, Whaddon Royston Herts (GB)

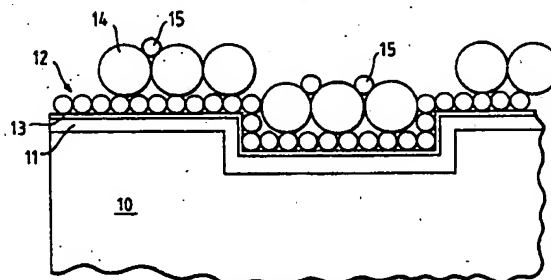
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74 Representative: Abrams, Michael John et al, HASELTINE LAKE & CO. Hazlitt House 28 Southampton Buildings Chancery Lane, London WC2A 1AT (GB)

54 Assay technique.

57 An assay technique is disclosed for the qualitative and/or quantitative detection of a chemical, biochemical or biological species in a sample. The technique comprises (a) coating at least a predetermined part of a pre-formed surface on a substrate with a thin film of a material capable of binding the species to be assayed, the pre-formed surface being optically active with respect to radiation at least over a predetermined band of wavelengths; (b) containing the coated surface with the sample; and (c) observing the optical properties of said pre-formed surface in order to determine a qualitative and/or quantitative change in optical properties as a result of the binding of the species onto said thin film of material. The optical properties of the pre-formed surface may be observed before and after step (b) in order to determine any change in optical properties, or they may be monitored during step (b). The pre-formed surface is preferably a grating.

An article for use in the above technique is also disclosed, and comprises a substrate carrying said pre-formed surface which in turn is coated with the receptive material for the species to be assayed.



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1                   "ASSAY TECHNIQUE"

          This invention relates to an assay technique for qualitative and/or quantitative detection of chemical, biochemical or biological species in a sample.

5           The technique is based upon the affinity of the species which is to be assayed for a receptive material, for example a ligand or a specific binding partner, which receptive material is coated onto a particular type of surface.

10           More particularly, according to one aspect of the present invention, there is provided an assay technique for qualitative and/or quantitative detection of a chemical, biochemical or biological species in a sample, which comprises (a) coating at least a predetermined part  
15 of a pre-formed surface on a substrate with a thin film of a material capable of binding the species to be assayed, the pre-formed surface being optically active with respect to radiation at least over a predetermined band of wavelengths; (b) contacting the coated surface  
20 with the sample; and (c) observing the optical properties of said pre-formed surface in order to determine a qualitative and/or quantitative change in optical properties as a result of the binding of the species onto said thin film of material.

25           In a first embodiment of the method of this invention, the optical properties of the pre-formed surface are observed before and after step (b) in order to determine any change in optical properties as a result of the species being bound by the receptive material in  
30 the thin film coating on the pre-formed surface. In a second embodiment, the optical properties of the pre-formed surface are monitored during step (b) in order to determine the said change in optical properties.

          According to a second aspect of the present  
35 invention, there is provided an article for use in an assay technique as defined above, which article comprises a substrate having a pre-formed surface which is

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1 optically active with respect to radiation at least over  
a predetermined band of wavelengths, and at least a  
predetermined part of which pre-formed surface is coated  
with a thin film of a material capable of binding a  
5 predetermined chemical, biochemical or biological  
species.

The pre-formed surface is preferably a grating. A  
single grating may be employed, or the surface may  
comprise two or more gratings disposed mutually at an  
10 angle. Where there are two such gratings, they may be  
mutually orthogonal. The profile of the or each grating  
is advantageously square-wave or sinusoidal. Saw-tooth  
profiles are also possible, but are not presently  
preferred.

15 The pre-formed surface may alternatively comprise  
a regular array of protuberances. With a surface of  
this type, the alignment of the peaks of the  
protuberances and the troughs between the protuberances  
corresponds to the ridges and troughs of a grating-type  
20 structure.

The thin film of receptive material may be coated  
onto the pre-formed surface so as to be deposited only in  
the troughs of the grating or in the troughs between the  
protuberances. A monomolecular layer of the receptive  
25 material will suffice and will generally be preferred,  
whether or not the coating is confined to the troughs.

The surface structure of the pre-formed surface  
and in particular the dimensions of the surface relief  
pattern will be selected according to the nature of the  
30 species which is to be assayed. In general, we have  
found three ranges of surface depth (peak-to-trough  
measurements) to be advantageous. In the first, the or  
each grating or the protuberances has or have a depth in  
the range 10 to 50 nanometers. In the second, the depth  
35 is in the range 50 to 200 nanometers; and in the third,  
the depth is in the range 200 to 2000 nanometers. With  
the first of these ranges, the pitch (period) of the or

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1 each grating or the periodicity of the protuberances is  
advantageously greater than their depth; the structure  
thus corresponds, in general, to that of a shallow  
grating. With the second and third ranges, the pitch  
5 (period) of the or each grating or the periodicity of the  
protuberances is advantageously of the same order as  
their depth.

In a first group of embodiments, the pre-formed  
surface is structured so that it is optically active with  
10 respect to radiation whose wavelength is in the range  
from 700 to 1500 nanometers. In a second group of  
embodiments, the pre-formed surface is structured so that  
it is optically active to radiation whose wavelength  
falls within the range from 350 to 700 nanometers.

15 Conveniently, the substrate which carries the  
pre-formed surface is formed of a plastics material.  
Plastics materials curable by ultra-violet light are  
preferred, and in particular acrylic or polyester  
materials can advantageously be used. A presently  
20 preferred plastics material is polymethylmethacrylate. A  
plastics substrate for use in this invention preferably  
has a refractive index in the range 1.25 to 1.6, and more  
preferably a refractive index of about 1.4.

An alternative substrate is a glass coated with a  
25 synthetic polymeric material.

The active surface of the substrate (i.e., that  
surface which is, or which carries, the pre-formed  
surface) can be constituted by a metal or a metal layer.  
Thus a plastics substrate, e.g., of polymethylmeth-  
30 acrylate, can have adhering thereto a metal layer which  
constitutes the pre-formed surface (e.g. a single grating  
structure of depth about 250 nanometers and period about  
400 nanometers). With such a structure, the  
plastics/metal interface may be planar, or it may conform  
35 to the surface structure of the metal layer itself. The  
metal used to form such layers may be gold, silver,  
copper or aluminium. Alternatively, the active surface

1 of the substrate may be constituted by an inorganic oxide  
or a layer thereof. The inorganic oxide is  
advantageously an oxide of silver, copper or aluminium.  
Such an oxide layer may be produced by causing or  
5 allowing oxidation of the surface of a metal substrate or  
of a metal layer adhering to a substrate of a different  
material. Where there is a layer of metal or of an  
inorganic oxide as just described, the layer is  
preferably from 5 to 50, more preferably 10 to 30,  
10 nanometers thick.

Conveniently, the substrate is lamellar and in  
strip-form. This facilitates use of an article in  
accordance with the invention in carrying out the assay.

15 The observation of optical properties in step (c)  
of the method of this invention can take place in  
transmission or in reflection. One zone of the  
pre-formed surface on the substrate may be left free of  
the coating of receptive material; the method may be  
performed by keeping this one zone free from sample, in  
20 step (b), or by contacting the whole of the pre-formed  
surface, including said one zone, with the sample. This  
latter technique has advantages in that any optical  
effects caused by components of the sample other than the  
species to be assayed will affect the coated and uncoated  
25 zones equally, and thus will cancel each other out when a  
comparison between the coated and non-coated zones is  
made. A two-beam illuminating system can be employed in  
step (c) of the method, one of the beams being directed  
at the uncoated zone of the pre-formed surface, and the  
30 other of the two beams being directed at a part of the  
coated zone of the pre-formed surface. Preferably,  
monochromatic radiation is used.

When the substrate and the pre-formed surface are  
constituted by a plastics material, and observations of  
35 the optical properties of the surface are to be carried  
out in transmission, it is preferred that the uncoated,  
pre-formed surface when viewed in transmission normal to

- 1 the plane of the surface should have a transmission not exceeding 1% for the radiation which is to be used.

In order to give optimum results when the technique of this invention is used for quantitative  
5 analysis, it may be advantageous to calibrate the coated substrate by first carrying out the assay technique using a sample containing a known proportion of the species which is to be assayed.

The present invention is applicable, for example,  
10 to testing a biological liquid, e.g. a blood sample, for specific antigen molecules. In such a case, the receptive material capable of binding the species to be assayed will comprise antibodies for the antigen concerned. Alternatively, it is possible to use an  
15 antigen as the receptive material and to assay a sample for antibodies. Where the receptive material comprises antibodies, these are preferably monoclonal antibodies. Antigens and antibodies occur in a wide range of molecular dimensions, and the surface structure of the  
20 pre-formed surface will be determined in part by the size of the molecules concerned. As an example, antigens resulting from many parasitic infections are typically in the size range from about 0.5 microns to 10 microns; for these antigens, a grating pitch of greater than 6 microns  
25 and preferably greater than 10 microns is desirable. In general, a grating pitch of the order of twice the antigen size will be desirable.

The invention is also applicable to the assaying of other chemical, biochemical or biological species, for  
30 example ionic species. The invention may be used, for example, to assay the metal ion content of a sample. The receptive material may be, for example, a chelating chemical or enzyme or a chelating organism which constitutes a specific binding partner for the ligand or  
35 ion which is to be assayed. In general, the enzyme or organism will be one or more of: a polypeptide, a steroid, a saccharide or polysaccharide, a proteoglycan,

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1 a nucleotide, a nucleic acid, a protonucleic acid, a  
microbial cell or a yeast.

Application of the invention thus lies not only in  
the medical field for diagnostics, but also generally in  
5 the field of process control.

The thin film of receptive material is preferably  
bonded firmly to the pre-formed surface of the substrate.  
Thus the receptive material may be bonded by  
electrostatic or covalent bonding to said surface.

10 Observations in step (c) of the method of this  
invention may use polarised light. In one particular  
technique, the pre-formed surface of the substrate is in  
the form of a single grating of square-wave or sinusoidal  
profile, and the optical properties of the surface are  
15 observed, in step (c), by monitoring the angular position  
at which there occurs a sharp reduction (dip) in  
reflection as the surface is observed or scanned with  
polarised radiation of a predetermined wavelength. The  
radiation used is preferably light, and the polarisation  
20 should be transverse to the grooves of the grating.

A presently preferred article in accordance with  
this invention consists of a profiled plastics strip,  
desirably fabricated by an embossing or casting  
technique, and with a refractive index of the order of  
25 1.4 and a transmission not exceeding 1%. The strip  
profile may be that of a single grating with square  
grooves, dimensioned for zero order suppression over a  
range of wavelengths. However, other profiles and  
dimensions can be used if desired, enabling diffraction  
30 efficiency into particular orders to be enhanced or  
suppressed.

An article in accordance with this invention may  
have a plurality of zones, each of which is coated with a  
different receptive material. In this way, a single  
35 article, e.g. in the form of a strip, can be used to  
assay a plurality of different species, e.g. antigens in  
a blood sample or metal ions in a biochemical fluid or in

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1 an industrial effluent.

In the case of a square profile grating, if the pitch is  $d$ , the groove height  $h$  and the refractive index  $n$ , then zero order diffracted light of wavelength  $W$  will  
5 be suppressed for  $h = W/2(n-1)$ , whilst first order diffracted light will emerge at angles given by  $\sin \theta = \pm W/d$ . For application to blood sampling, given a grating pitch of about 6 microns, and a source wavelength of 550 nm (green), then  $h = 0.69$  microns;  $\theta = \pm 5.2^\circ$ .

10 The principle of the assaying method is that the receptive material, e.g. antibodies, coated on the grating are typically small molecules, e.g. sized around 10 nm, and are too small to produce any size or shape dependent light scattering. However, the antigens  
15 attached to the antibodies when a blood sample is smeared on the grating have a size of the same order as the wavelength of incident light, and have an effect analogous to that of filling some of the grating grooves with water (refractive index 1.33). This means that, in  
20 the case of a grating dimensioned as above, zero order light is no longer suppressed, whilst very little light is diffracted into the higher orders. Generally, therefore, the transmission of the grating, normally not exceeding 1%, will be directly related to the number of  
25 antigens present.

The method thus depends on determination of the change in optical properties, e.g. transmission or reflection characteristics, of the grating. For this reason, given a grating coated with antibodies over its  
30 whole area, the smearing of a part of this area with the sample can readily enable the said change to be quantitatively determined. A similar effect is preferably achieved, however, by coating only a part of the grating with antibodies, as the antigens will not be attracted  
35 into and trapped in the grooves in the uncoated region. Preferably, in conjunction with the last mentioned partly coated grating, a two beam illuminating system will be



1 employed. The source may be an incandescent lamp  
emitting light incident on the grating through a filter.  
The angle of incidence of the monochromatic (or nearly  
monochromatic) light on the grating is preferably 0°  
5 (i.e. normal to the grating) and, for the grating  
exemplified above, zero order diffracted light would be  
collected by means of a lens onto a photodetector, while  
higher order diffracted light would be obscured using a  
stop.

10 One aim of the invention is to provide a low cost  
pre-coated grating which can be widely used for  
diagnostic purposes, commonly in a general practitioner's  
surgery but possibly also in the home. For this  
purpose, the antibodies would be firmly bonded to the  
15 plastics grating, e.g. by electrostatic bonding which can  
ensure virtually permanent coating provided that a  
suitable or suitably treated plastics material is  
initially chosen to form the grating. As the aim would  
usually be to detect a specific antigen, the grating  
20 would be coated with a specific antibody, e.g. a  
monoclonal antibody which attracts and retains only the  
specific antigen in question. Thus, successive testing  
of a plurality of selectively coated gratings would  
enable quantitative detection of specific antigens as an  
25 aid to diagnosis.

The technique of smearing the grating with the  
sample also requires consideration. After wiping the  
grating with, say, a blood sample, it is important to  
remove any excess sample in order to ensure that minimum  
30 carrier liquid, minimum haemoglobin and minimum large  
cells other than antigen are retained.

As the effect of absorption by red cells  
containing haemoglobin can be minimised by suitable  
choice of the wavelength of illumination, it is the  
35 retention of carrier liquid which is the next likely  
source of errors of detection. For minimising such  
errors, the grating may be dimensioned for zero order

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1 suppression when there is a continuous liquid film on top  
of the grating; this requires a modified grating height  
of  $h = W/2 (n_1 - n_2)$ , where  $n_1$  is the refractive index of  
the substrate and  $n_2$  is the refractive index of the  
5 liquid. A liquid of high refractive index is desirable,  
and one suitable example is glycerol. The smearing  
technique (i.e. step (b)) would then include the step of  
washing the grating, after wiping it with the sample,  
with the liquid in question.

10 A further point to be understood in connection  
with the smearing technique is that this will commonly  
result in only a small percentage, e.g. less than 2%, of  
the overall area of the grating bearing and retaining  
attracted antigens. The use of a diffractor grating of  
15 high sensitivity relieves the illuminating and detector  
system of the extreme requirements which would otherwise  
be required quantitatively to detect such a small  
presence of antigen, thus making practical the use of  
relatively simple and low cost optics which can enable  
20 widespread use.

One example of assaying method and apparatus in  
accordance with the invention is shown in the  
accompanying drawing, in which:

FIGURE 1 shows an optical illuminating and  
25 detecting system;

FIGURE 2 shows a detail of one embodiment of an  
article incorporating a diffraction grating and forming  
part of the system of Figure 1;

FIGURE 3 shows a cross-sectional view (not to  
30 scale) of a second embodiment of an article incorporating  
a diffraction grating; and

FIGURE 4 illustrates the results obtained with an  
article of the type illustrated in Figure 3.

Referring first to Figure 2, a square profile  
35 single diffraction grating 10 whose pitch and depth are  
both equal to 800 nanometers is coated with a  
substantially mono-molecular layer of immobilised

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1 antibodies 12, preferably monoclonal antibodies. After  
smearing with a sample, an antigen 14, being a binding  
partner to the antibodies, is attracted and trapped in  
one groove. At this point of the grating, the zero  
5 order diffracted light is transmitted, as indicated at  
16, instead of being suppressed.

Figure 1 shows the grating 10 under illumination  
by monochromatic light 18. Zero order diffracted light  
is collected by a lens 20 onto a photodetector 22, while  
10 higher order diffracted light is obscured by a stop 24.  
A two-beam illuminating system which, as described above,  
is generally preferred, will operate in a precisely  
analogous way.

Referring next to Figure 3, an article in  
15 accordance with this invention is shown in the condition  
after it has been contacted by a sample in step (b) of  
the method of the invention. The article comprises a  
substrate 10 formed of polymethylmethacrylate which is  
about 1 millimeter thick. The active (upper) surface of  
20 the substrate includes a layer 11 of aluminium of  
thickness 20 nanometers. This is covered by a passive  
film 13 of aluminium oxide (thickness one nanometer or  
less). A monomolecular layer of antigen molecules 12 is  
covalently bonded to the film 13 of aluminium oxide and  
25 is thus immobilised. A layer of antibodies 14 is  
attached to the antigen layer 12. This layer 14 is also  
monomolecular and is about ten nanometers thick.  
Isolated antigens 15 have been bound by the antibodies  
14. The substrate 10 with the layers 11, 13, 12 and 14  
30 constitutes one embodiment of the article of this  
invention. The pre-formed surface is in effect defined  
by the surface of layer 13; this is in the form of a  
single grating of depth 50 nanometers and of pitch  
(period) 250 nanometers. The article is observed, in  
35 carrying out the method of the invention, with  
monochromatic light which is polarised in a plane  
perpendicular to the lines of the grating; the angle of

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1 incidence of the illumination is varied and it is found  
that there is a sharp reduction (dip) in reflectivity at  
an angle whose value depends upon the amount of material  
(antibodies 14) overlying the article. The angular  
5 position of this dip, and also its angular width, are  
strongly dependent upon the amount of antigens attached  
to the layer 14 of antibodies and hence these parameters  
provide a quantitative measure of the antibodies absorbed  
from the sample. Figure 4 plots the reflectivity of the  
10 article against the angle of incidence of the  
monochromatic, polarised illumination over a small  
angular range. As the quantity of antigens captured by  
the antibody layer 14 increases, the dip in reflectivity  
first of all becomes more pronounced, and then becomes  
15 broader and the angular position of the reflectivity  
minimum alters, as shown in the three curves plotted.  
The reflectivity dip can be considered theoretically as a  
plasmon resonance; it is relatively easy to detect a  
change in the angle of incidence of about 0.1 degrees or  
20 a change in the wavelength of the resonance by about 1  
nanometer. Hence it is possible to detect changes  
corresponding to an increase in the average thickness of  
the antigen layer 15 of around one nanometer. It will  
be appreciated that, when antigens are bound by the layer  
25 14, the result is not the addition of a further layer of  
uniform thickness; nevertheless, we have found that the  
occurrence of isolated antigens 15 over the layer 14 of  
antibodies behaves approximately as though they were  
"smoothed out" into a layer whose average thickness  
30 modifies the optical properties of the system as a whole.

1 Claims:

1. An assay technique for qualitative and/or quantitative detection of a chemical, biochemical or biological species in a sample, which comprises (a) coating at least a predetermined part of a pre-formed surface on a substrate with a thin film of a material capable of binding the species to be assayed, the pre-formed surface being optically active with respect to radiation at least over a predetermined band of wavelengths; (b) contacting the coated surface with the sample; and (c) observing the optical properties of said pre-formed surface in order to determine a qualitative and/or quantitative change in optical properties as a result of the binding of the species onto said thin film of material.

2. A method according to claim 1, wherein the optical properties of said pre-formed surface are observed before and after step (b) in order to determine the said change in optical properties.

3. A method according to claim 1, wherein the optical properties of said pre-formed surface are monitored during step (b) in order to determine the said change in optical properties.

4. A method according to claim 1, 2 or 3, wherein said pre-formed surface is a grating.

5. A method according to claim 1, 2 or 3, wherein said pre-formed surface comprises two or more gratings disposed mutually at an angle.

6. A method according to claim 4 or 5, wherein the or each grating is of square-wave profile.

7. A method according to claim 4 or 5, wherein the or each grating is of sinusoidal profile.

8. A method according to claim 4 or 5, wherein the or each grating is of saw-tooth profile.

9. A method according to claim 1, 2 or 3, wherein said pre-formed surface comprises a regular array of

1 protuberances.

10. A method according to claim 4, 5, 6, 7, 8 or 9, wherein said thin film of material is coated onto the pre-formed surface so as to be deposited only in the  
5 troughs of the grating or in the troughs between the protuberances.

11. A method according to any one of claims 4 to 10, wherein the or each grating or the protuberances has or have a depth (peak-to-trough) in the range 10 to 50  
10 nanometers.

12. A method according to any one of claims 4 to 10, wherein the or each grating or the protuberances has or have a depth (peak-to-trough) in the range 50 to 200 nanometers.

13. A method according to any one of claims 4 to 10, wherein the or each grating or the protuberances has or have a depth (peak-to-trough) in the range 200-2000 nanometers.

14. A method according to claim 12 or 13, wherein  
20 the pitch (period) of the or each grating or the periodicity of the protuberances is of the same order as their depth.

15. A method according to claim 11, wherein the pitch (period) of the or each grating or the periodicity  
25 of the protuberances is greater than their depth.

16. A method according to any one of claims 1 to 10, wherein said pre-formed surface is structured so that it is optically active with respect to radiation of wavelengths from 700 to 1500 nanometers.

17. A method according to any one of claims 1 to 10, wherein said pre-formed surface is structured so that it is optically active to radiation of a wavelength in the range from 350 to 700 nanometers.

18. A method according to any preceding claim,  
35 wherein the substrate is formed of a plastics material.

19. A method according to claim 18, wherein said plastics material is a material which is curable by

1 ultra-violet light.

20. A method according to claim 18 or 19, wherein said plastics material is an acrylic or a polyester material.

5 21. A method according to claim 20, wherein said plastics material is polymethylmethacrylate.

22. A method according to any one of claims 1 to 17, wherein the substrate is a glass coated with a synthetic polymeric material.

10 23. A method according to any one of claims 1 to 17, wherein at least the active surface of the substrate is constituted by a metal or a metal layer.

24. A method according to any one of claims 1 to 21, wherein the active surface of the substrate is  
15 constituted by an inorganic oxide or a layer thereof.

25. A method according to claim 23, wherein said metal is gold, silver, copper or aluminium.

26. A method according to claim 24, wherein said inorganic oxide is an oxide of silver, copper or  
20 aluminium.

27. A method according to any preceding claim, wherein the substrate is in strip-form.

28. A method according to claim 18, 19, 20 or 21, wherein the plastics material has a refractive index in  
25 the range 1.25 to 1.6.

29. A method according to claim 28, wherein the refractive index of said plastics material is about 1.4.

30. A method according to any preceding claim, wherein in step (c) the optical properties of said  
30 pre-formed surface are observed in transmission.

31. A method according to any one of claims 1 to 29 wherein in step (c) the optical properties of said pre-formed surface are observed in reflection.

32. A method according to any preceding claim,  
35 wherein one zone of the pre-formed surface on the substrate is left free of the coating material and is not contacted, in step (b), by the sample.

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1           33. A method according to any one of claims 1 to  
31, wherein one zone of the pre-formed surface is left  
free of the coating material and the whole of the  
pre-formed surface, including said one zone, is  
5 contacted, in step (b), by the sample.

          34. A method according to claim 32 or 33, wherein  
a two-beam illuminating system is employed in step (c),  
one of said beams being directed at said one zone of the  
pre-formed surface, and the other of the two beams being  
10 directed at a part of said pre-formed surface other than  
said one zone.

          35. A method according to any preceding claim,  
wherein in step (c) monochromatic radiation is used.

          36. A method according to any preceding claim,  
15 wherein the species which is to be detected is an  
antigen.

          37. A method according to claim 36, wherein the  
material capable of binding said species comprises  
antibodies for the antigen which is to be assayed.

20           38. A method according to claim 37, wherein said  
antibodies are monoclonal antibodies.

          39. A method according to any one of claims 1 to  
36, wherein the species which is to be assayed is an  
ionic species.

25           40. A method according to claim 39, wherein said  
ionic species is a metal ion.

          41. A method according to claim 39 or 40, wherein  
the material capable of binding the species to be assayed  
is a chelating enzyme or a chelating organism.

30           42. A method according to claim 41, wherein said  
enzyme or organism is one or more of: a polypeptide, a  
steroid, a saccharide or polysaccharide, a proteoglycan,  
a nucleotide, a nucleic acid, a protonucleic acid, a  
microbial cell or a yeast.

35           43. A method according to any preceding claim,  
wherein said thin film of material is firmly bonded to  
the pre-formed surface of the substrate.



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1           44. A method according to claim 43, wherein said  
thin film of material is bonded to the pre-formed surface  
of the substrate by electrostatic or covalent bonding.

5           45. A method according to any preceding claim,  
wherein, in step (c), polarised light is used to observe  
the optical properties of the pre-formed surface.

10           46. A method according to claim 45, wherein the  
pre-formed surface of the substrate is in the form of a  
single grating of square-wave or sinusoidal profile, and  
wherein the optical properties of the pre-formed surface  
are observed, in step (c), by monitoring the angular  
position at which there occurs a sharp reduction (dip) in  
reflection as the surface is observed or scanned with  
radiation of a predetermined wavelength.

15           47. An article for use in an assay technique as  
claimed in claim 1, which comprises a substrate having a  
pre-formed surface which is optically active with respect  
to radiation at least over a predetermined band of  
wavelengths, and at least a predetermined part of which  
20 pre-formed surface is coated with a thin film of a  
material capable of binding a predetermined chemical or  
biochemical or biological species.

          48. An article as claimed in claim 47, wherein  
the substrate is a plastics material.

25           49. An article as claimed in claim 48, wherein  
said plastics material is a material which is curable by  
ultra-violet light.

          50. An article as claimed in claim 48 or 49,  
wherein said plastics material is an acrylic or a  
30 polyester material.

          51. An article as claimed in claim 50, wherein  
said plastics material is polymethylmethacrylate.

35           52. An article as claimed in claim 47, wherein  
the substrate is a glass coated with a synthetic  
polymeric material.

          53. An article as claimed in any one of claims 47  
to 52, wherein the substrate is lamellar.

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1        54. An article as claimed in claim 53, wherein  
the substrate is in strip-form.

5        55. An article as claimed in claim 52, 53 or 54,  
wherein the pre-formed surface is in the form of a single  
grating or of two or more gratings disposed mutually at  
an angle.

56. An article as claimed in claim 55, wherein  
the or each grating is of square-wave, sinusoidal or  
saw-tooth profile.

10       57. An article as claimed in claim 52, 53 or 54,  
wherein the pre-formed surface comprises a regular array  
of protuberances.

15       58. An article as claimed in any one of claims 47  
to 57, wherein said pre-formed surface is constituted by  
a metal or a metal layer.

59. An article as claimed in claim 58, wherein  
said metal is gold, silver, copper or aluminium.

20       60. An article as claimed in any one of claims 47  
to 59, wherein said pre-formed surface is constituted by  
an inorganic oxide.

61. An article as claimed in claim 60, wherein  
said oxide is an oxide of silver, copper or aluminium.

25       62. An article as claimed in any one of claims 47  
to 61, wherein said thin film of material comprises  
antibodies.

63. An article as claimed in claim 62, wherein  
said antibodies are monoclonal antibodies.

30       64. An article as claimed in any one of claims 47  
to 61, wherein said thin film of material comprises a  
chelating enzyme or a chelating organism.

65. An article as claimed in claim 48, wherein  
the substrate has a refractive index in the range 1.25 to  
1.6.

35       66. An article as claimed in claim 65, wherein  
the refractive index of the substrate is about 1.4.

67. An article as claimed in claim 48, wherein  
the pre-formed surface of the substrate when viewed in

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1 transmission normal to the plane of the pre-formed  
surface has a transmission not exceeding 1%.

68. An article as claimed in claim 58 or 59,  
wherein the pre-formed surface is constituted by a layer  
5 of thickness in the range 5 to 50 nanometers.

69. An article as claimed in any one of claims 47  
to 68, wherein the article includes a plurality of zones  
each of which is coated with a different receptive  
material so that the article is capable of binding a  
10 plurality of different species.

70. A method according to claim 1, in which the  
pre-formed surface is washed immediately after being  
contacted with the sample and before the observations in  
step (c)..

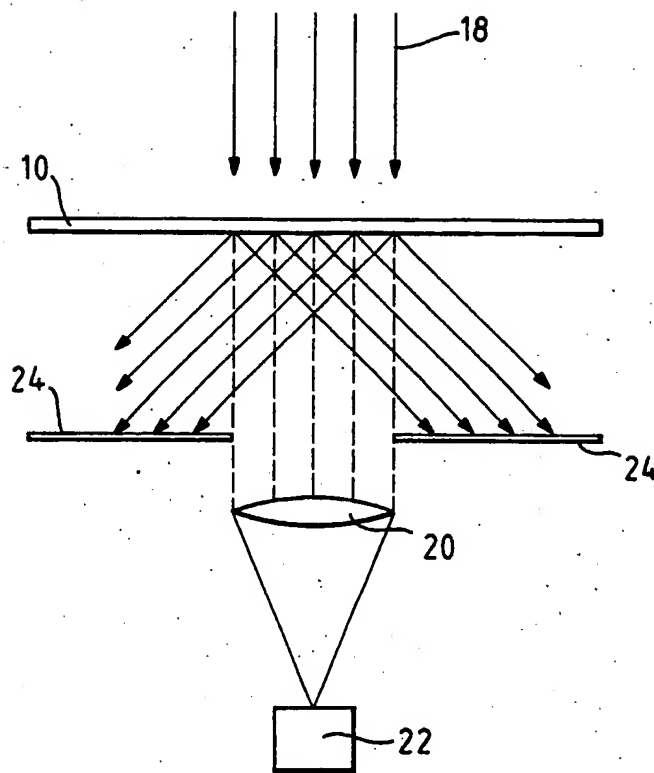
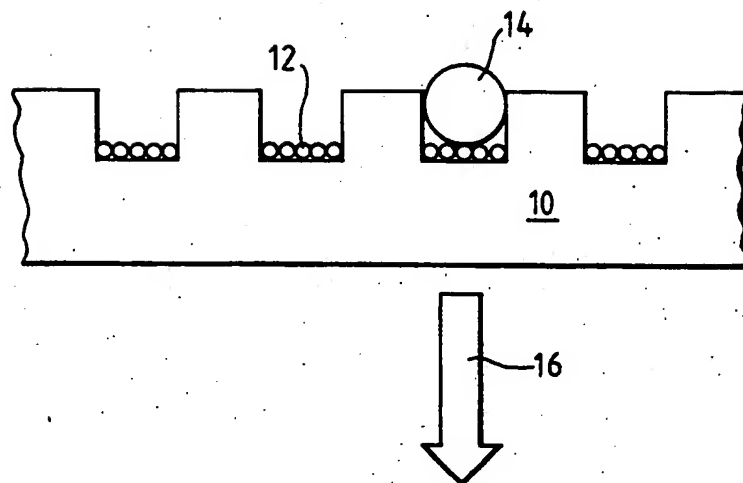
15 71. A method according to claim 1 to 70, in which  
the pre-formed surface is covered with a layer of a  
liquid of high refractive index between steps (b) and  
(c).

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*Fig. 1.**Fig. 2.*

A cross-sectional view of a semiconductor device structure. A substrate 10 is shown with a top surface 11. A layer 12 is formed on the top surface 11. A series of small circles 13 are arranged in a row on the layer 12. Above the small circles 13, there are three large circles 14 and two small circles 15. The structure is shown in a cross-section with a central recessed area.

The graph shows reflectivity on the y-axis and angle of incidence on the x-axis. A horizontal line represents the reflectivity for normal incidence. Three curves are shown: a solid curve that dips below the normal incidence line, and two dashed curves that remain above it. All curves show a minimum reflectivity at a specific angle of incidence.